

# Effect of Metals on Mutagenesis and DNA Repair

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Unlike the situation with organic compounds, metals do not show a high correlation between carcinogenicity and mutagenicity. An agent may be mutagenic by causing misreplication of DNA due to alterations of the DNA template, decreased fidelity of DNA polymerase, or inhibition of the proofreading of DNA replication. In addition, bacteria have an inducible, error-prone DNA repair system (SOS repair) whose activity results in mutagenesis. In the best studied example of metal mutagenesis, chromate, there is little evidence for the involvement of the SOS system. Metals may act as comutagens by inhibiting the repair of damage to DNA caused by another agent. This has been demonstrated for arsenite. Comutagens would not be detected by standard screening methods.

## Introduction

Most organic carcinogens or their metabolites have been shown to bind to DNA (1). In many cases, specific adducts have been identified. Although DNA repair mechanisms can remove much of this damage, some adducts have been shown to persist for many generations (2). That persistent damage to DNA leads to carcinogenesis is suggested by the human genetic disease Xeroderma pigmentosum. Patients with this disease have defects in the repair of UV-induced pyrimidine dimers, and multiple skin cancers arise on parts of the body exposed to sunlight. A recent review of this disease has been published by Setlow (3).

The realization that many carcinogens derive their activities from their abilities to react with DNA has led to the development of a number of short-term tests based on mutagenicity. Because of their simplicity, sensitivity, economy, and short time scale, bacterial systems have been useful for studies on the mutagenicity of carcinogens. With some exceptions, agents which are mutagenic to bacteria are also mutagenic to animal cells. Metal

mutagenicity studies have been carried out almost exclusively in bacterial systems, although some studies on the effects of metals on animal cell chromosomes have been carried out. Chromosomal abnormalities are discussed in a separate paper on that subject (4), and only gene mutations will be discussed in this paper.

Metal mutagenesis has been reviewed by Flessel (5). In 1951, manganese was shown to be a bacterial mutagen (6). Since that time chromate (Cr VI) has been established as a mutagen in a variety of bacterial systems. Other metal compounds reported to mutagenize *S. typhimurium* include ferrous sulfate, *cis*-diamminoplatinum tetrachloride, and selenate but not selenite. Negative results were reported for arsenite and arsenate (5). In *E. coli*, molybdenate and arsenite have been reported as mutagens (7). However, attempts by this author to demonstrate mutagenesis by arsenite, using a variety of protocols, yielded only negative results (8). Negative results in the *E. coli* system were reported for compounds of tungsten, molybdenum, zinc, cadmium and mercury (5).

In general, the strains of bacteria used for mutagenesis testing of metals have given either inconsistent results, or results which do not correlate well with the carcinogenicity of the metals. It has been known for some time that the Ames test does not predict well for metals suspected or known

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to be carcinogenic (9). The Ames tester strains have been developed for increased sensitivity toward mutagens which form bulky lesions on DNA and work via an error-prone DNA repair pathway (10). As will become clear in this review, a number of other mechanisms by which metals could be mutagenic exist. In addition, some metals might act as comutagens rather than as primary mutagens, and a different test procedure is needed to demonstrate comutagenesis.

## Mechanisms of Mutagenesis

Drake and Baltz (11), in a review of biochemical mechanisms of mutagenesis, have divided mutagenic mechanisms into two major classes. Class I involves directly induced base mispairing, whereas Class II applies to agents which interrupt normal DNA replication by preventing base-pairing of any kind at the damaged site, and mutations result as errors in DNA repair. Since there are many mechanisms by which direct base mispairing can occur, and since error-prone DNA repair has not been proven to occur in animal cells, I shall use a different method of classification based on possible sites at which metals might act in causing mutations.

### Misreplication Due to Altered DNA

Some of the earliest examples of analysis of mutagenic mechanisms were performed on agents which chemically alter the DNA (11). For example, nitrous acid, which deaminates cytosine to uracil and adenine to hypoxanthine, generates point mutations. More recently, some alkylating agents, such as ethyl methane sulfonate, have been shown to cause direct base mispairing due to the formation of alkylation products on the purine and pyrimidine oxygens (12). The mutagenicity of bisulfite ( $\text{SO}_2$  in solution) is due to the deamination of cytosine to uracil (13).

In order for metals to cause mutations by this mechanism, the metal must either bind to DNA in such a way as to cause base mispairing during DNA replication, or it must cause a chemical alteration of the DNA by another mechanism. The binding of metals to nucleic acids has been reviewed by Sundaralingam (14) and Eichhorn (15). In general, metal complexes affect neither the nucleotide geometry nor their conformations. Exceptions are the alterations in bondlengths and angles in cadmium-GMP complex and in a few other cases where N(7) is the sole site of a transition metal binding. Also, some platinum complexes contain the rare trans C(4')-C(5') conformation in the sugar.

Metals can bind to bases, phosphate groups, or sugars in nucleotides. Phosphate is the strongest coordinating group for most metals. In general, the stability of metal compounds to nucleosides reflects the stability of binding to phosphate (15). Lesions on the phosphate groups or sugars of DNA are assumed to be of little biological consequence unless gross distortions of the DNA helix result. Metals which bind strongly to phosphates tend to stabilize the DNA helix (increase in  $T_m$ ). The order of binding to phosphates in preference to bases is  $\text{Mg (II)} > \text{Co (II)} > \text{Ni (II)} > \text{Mn (II)} > \text{Zn (II)} > \text{Cd (II)} > \text{Cu (II)}$ .

The purine bases exhibit higher reactivity towards metal ions than do the pyrimidines. The ring nitrogen of purines is favored over the amino nitrogens or keto oxygen. The N(7) position of purines is the favored binding site for  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Mn}^{2+}$ , while  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  tend to bind to N(3) of cytosine and N(1) of adenine. It should be noted that the N(7) position of purines is not involved in base pairing and its alteration would not be expected to have mutagenic consequences. No information was available on the binding sites of compounds of arsenic, chromium, selenium or beryllium to nucleotides or polynucleotides.

Metal compounds might also form cross-links in DNA. The preferred binding of  $\text{Hg}^{2+}$  to alternating poly d(A-T) involves cross linking to two thymine residues. A model for the binding of *cis*-diammino platinum suggests binding to the N(7) atoms of two adjacent purines on the same strand.

A great deal of work needs to be done in the area of metal DNA complexes, with particular attention to carcinogenic metals. The biological consequences of metal binding to DNA needs to be examined as well. Many carcinogenic metals have been shown to cause infidelity in DNA replication (16). The mechanism of this effect could be via metal interactions with DNA polymerase, or via metal interactions with DNA itself. If the binding of the metal to DNA or a synthetic polynucleotide is tight enough, these mechanisms could be distinguished by binding of the metal to the template and washing away of free metal prior to the misincorporation assay.

Indirect evidence for damage to DNA can be obtained by comparing the toxicities of metal compounds in strains of bacteria which are proficient in DNA repair and in strains defective in some DNA repair pathway. This is the basis for the Pol test and the rec assay (17, 18). A number of metal compounds have been tested in the latter, which compares toxicities in  $\text{rec}^+$  and  $\text{rec}^-$  strains of *B. subtilis*. Positive results (greater toxicity in  $\text{rec}^-$ ) were reported for:  $\text{AsCl}_3$ ,  $\text{NaAsO}_2$ ,  $\text{Na}_3\text{AsO}_4$ ,  $\text{K}_2\text{CrO}_4$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ ,  $\text{CH}_3\text{HgCl}$ ,  $\text{CH}_3\text{COOHgC}_6\text{H}_5$ ,  $\text{MnCl}_2$ ,

$\text{Mn}(\text{NH}_3)_2$ ,  $\text{MnSO}_4$ ,  $\text{Mn}(\text{CH}_3\text{COO})_2$  and  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ . More work should be done to correlate biochemical studies on alterations to DNA by metal compounds with studies on enhanced toxicity of metals to DNA repair-deficient bacteria.

In animal cells, indirect evidence for damage to DNA by metal compounds might be obtained by studying the recovery of DNA synthesis after removal of an inhibitory dose of the metal. As pointed out by Painter (19), DNA-damaging agents can be distinguished from other agents which inhibit DNA replication by the continued inhibition of DNA replication after the removal of the DNA damaging agent. In contrast, agents which inhibit DNA replication by another mechanism usually show an immediate recovery of DNA replication upon their removal. In this system, 5mM  $\text{NiCl}_2$  was inhibitory to DNA replication, but since recovery upon its removal was rapid, it did not behave like a DNA damaging agent. It would be of interest to see how other metal compounds behave in this system.

To summarize, a number of metal compounds have been shown to bind to purines and pyrimidines, and a number of compounds have been shown to have greater toxicity in DNA repair-deficient mutants of bacteria. Thus far, there is no clear correlation between binding to DNA and mutagenicity. No particular DNA-metal complex has been demonstrated as a premutational lesion, and there is no information as to other types of DNA damage, such as deamination, which might be caused by metals.

## Misreplication Due to Decreased Fidelity of DNA Polymerase

Errors in replication may be caused by agents which decrease the fidelity of DNA replication by affecting the DNA polymerase directly, rather than by damaging the DNA template. The effects of metals on the fidelity of DNA replication will be covered in the paper on infidelity of DNA synthesis.

## Inhibition of Proofreading

Prokaryotic DNA polymerases contain a 3'-5' exonuclease function which acts to excise newly incorporated (3'-terminal) mis-matched nucleotides. This is known as the proofreading function of the polymerase. A model for mutagenesis via alterations in proofreading comes from studies on phage  $\text{T}_4$  mutators (mutants which exhibit a higher than normal frequency of spontaneous mutations). Mutators of  $\text{T}_4$  sometimes have a DNA polymerase with low 3'-5' exonuclease to polymerase ratios,

which results in leaving too many mismatched bases in DNA (11). There is evidence that the carcinogenic metal beryllium can specifically inhibit the 3'-5' exonuclease function (20). The carcinogen azathioprine has also been reported to act in this manner (21).

However, in one study of the effects of metals on *E. coli* DNA polymerase I, the 3'-5' exonuclease function of the polymerase was not inhibited by metal salts at concentrations which caused a loss of fidelity of the polymerase (22).

Eukaryotic DNA polymerases do not contain a 3'-5' exonuclease function. It is possible that proof-reading is carried out by a separate enzyme. Evidence for such an activity has been reported (23). This question must be resolved before the effects of metal compounds on proofreading can be determined in eukaryotic cells.

## Mutagenesis via Error-Prone DNA Repair

In bacteria, some agents have been shown to cause mutations only when an error-prone DNA repair system (SOS system) is induced. Agents which are mutagenic by this mechanism are those which cause lesions on DNA which interrupt normal DNA replication by preventing base-pairing of any kind at the damaged site. Strictly speaking, this system is not really a repair system since lesions on DNA are not removed.

The best studied example of an agent which is mutagenic via the SOS system is ultraviolet light (24). Mutagenesis after UV-irradiation in *E. coli* requires the *recA*<sup>+</sup> and *lexA*<sup>+</sup> gene products and protein synthesis. It has been suggested that one of the induced proteins might alter DNA polymerase activity, allowing DNA replication past a lesion which previously had constituted a block to replication (e.g. a pyrimidine dimer). Since lesions of this sort are noncoding, nucleotides inserted opposite them must be random, and therefore a high probability for mutagenesis exists (25).

In bacteria with an SOS system, an agent which inhibits either the induction or action of this system will behave as an antimutagen. A few years ago, my coworkers and I reported such an effect for arsenite (26, 27). If *E. coli* is exposed to 1mM sodium arsenite after UV irradiation, both survival and mutagenesis are decreased. The most likely explanation for this effect is the inhibition of induction of the SOS system.

If an agent causes mutations solely via the SOS system, the agent will be unable to mutate strains of bacteria which have genetic defects in the SOS

system (i.e., *recA*<sup>-</sup> or *lexA*<sup>-</sup>). Based on studies in strains of *E. coli*, such a mechanism of action has been proposed for NaAsO<sub>2</sub>, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> (7). However, others were unable to demonstrate mutagenesis by arsenite in *E. coli* (8) or in *Salmonella* (28) or by salts of molybdenum in *E. coli* (29). In the case of chromate, Venit and Levy found mutagenesis in a *lexA*<sup>-</sup> (*exrA*<sup>-</sup>) strain (29). Thus, chromate mutagenicity probably does not occur via the SOS system in bacteria.

The existence of an SOS-like system in eukaryotic cells is controversial. The best evidence comes from studies on the enhanced survival of irradiated viruses when grown on cells which have previously been UV- or x-irradiated (30), or treated with low doses of carcinogens (31). There is also evidence that this repair is error-prone (32). However, another interpretation of this phenomenon has been presented (33). It has been suggested that the mechanisms by which mutations occur in eukaryotic cells are constitutive, in contrast to the inducible systems in prokaryotic cells (34, 35). Thus, it is premature to speculate about the effects of metals on a system which may not exist in eukaryotic cells.

## Effects on DNA Repair Leading to Comutagenesis

The function of DNA repair systems is to restore the informational content of DNA which has been damaged. With the exception of the SOS system described above, DNA repair processes generally suppress mutagenesis, i.e., most DNA repair is relatively error-free. The high incidence of skin cancers in patients with xeroderma pigmentosum suggests that unrepaired damage to DNA can also lead to carcinogenesis. Thus, any agent which interferes with (error-free) DNA repair is likely to act as a comutagen and a cocarcinogen.

Most of the discussion which follows will concern excision repair pathways. In bacteria, a number of post-replication repair (or recovery) systems exist whose function is to fill in daughter strand gaps opposite lesions, which are thought to arise due to blockage of replication at the lesion and resumption further on. These gaps may be filled in by a recombinational process or via the SOS system described above (11, 24, 25, 33, 36, 37). In eukaryotic cells (as in prokaryotes), DNA made immediately after UV irradiation has a smaller molecular weight than normal. With time, the molecular weight enlarges until a normal size is seen, suggesting the existence of daughter strand gaps and subsequent filling of the gaps. There is no evidence for recombinational repair in eukaryotic cells. A model

for post-replication repair in eukaryotic cells, in which gaps are filled by *de novo* DNA synthesis, has been presented. More recently, a number of other models and re-interpretations of data have been suggested (33, 36-39). Because of the confusion in the field of eukaryotic post-replication repair, in which its very existence is in doubt (33), it would be premature to discuss the effects of metal compounds on this system.

## Excision Repair

Excision repair of damaged DNA involves removal of a piece of DNA containing the damage and resynthesis (repair replication), using the complementary strand as template. The two major pathways of excision repair (Fig. 1) differ in the initial steps prior to repair replication. UV-induced pyrimidine dimers and large carcinogen-DNA adducts are repaired by a pathway known as nucleotide excision repair, in which the first step is incision of the damaged DNA by an endonuclease which recognizes the damage and cleaves the phosphodiester bond near the damage. The other major pathway is base excision repair. Here, such damage as uracil in DNA (which can result from deamination of cytosine), hydrated and ring-saturated bases, and small adducts are recognized by specific *N*-glycosylases, which cleave the *N*-glycosyl bond between the base and the sugar, leaving an apurinic or apyrimidinic (AP) site. An endonuclease which recognizes AP sites then performs an endonucleolytic cleavage. In both major pathways, polymerase, exonuclease and ligase action forms a patch of new DNA (repair patch). In animal cells, nucleotide excision repair is thought to result in larger patches than does base excision repair, suggesting that perhaps the exonuclease steps of these two repair systems are not identical. A more detailed discussion of excision repair is given elsewhere (33, 36, 37).

Evidence suggests that excision repair in prokaryotes and eukaryotes is an error-free process. Since the template strand is undamaged, repair replication should be as faithful as DNA replication itself. Mutants of bacteria which are defective in excision repair tend to be more readily killed and mutated by agents whose damage is not repaired (24, 36). Damaged DNA is more likely to have lethal and mutagenic consequences if the damage persists to replication than if it undergoes excision repair. The demonstration that the mutation frequency in UV-irradiated human fibroblasts is decreased when cells are kept in a confluent state after irradiation (where excision repair can occur but DNA replication cannot) is taken as evidence that excision repair in human cells is also an

## Two Major Pathways for Excision Repair

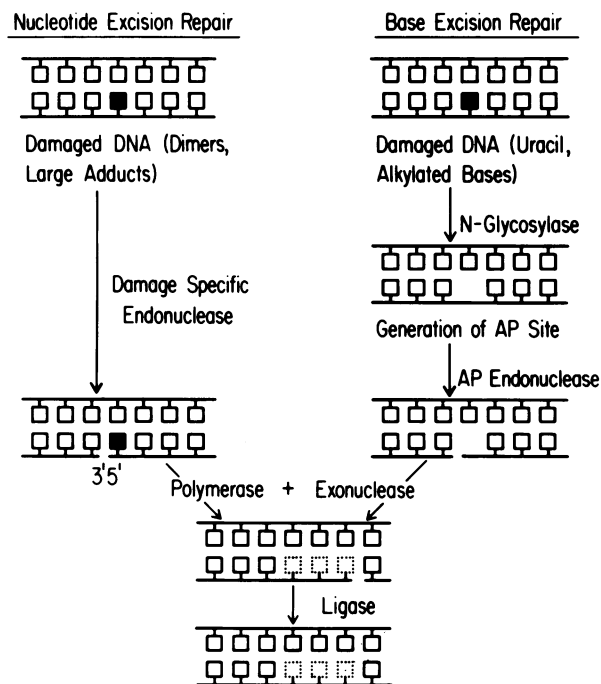


FIGURE 1. Pathways for excision repair: (□) normal bases; (■) damaged bases; (◻) repaired bases.

error-free process (40).

The effects of metal compounds on excision repair systems can be assayed by growing cells in the presence and absence of a nontoxic concentration of the metal compound after exposure of the cells to a DNA-damaging agent, and scoring for survival and mutagenesis. Since there are a variety of enzymes which recognize damage to DNA, agents which cause different types of damage should be tested. If the metal compound inhibits excision repair, an enhancement of the mutation frequency (comutagenesis) and decrease in survival should be seen. In bacterial systems, strains deficient in excision repair will not show this effect. Biochemical assays can be used to pinpoint the steps in repair which are affected. If the repair enzymes have been identified, direct assays of the effects on these enzymes by metal compounds can be carried out.

My laboratory has recently found that low concentrations of arsenite (but not arsenate) act as a comutagen with ultraviolet light in *E. coli* (41). When a *uvrA* mutant (which cannot excise pyrimidine dimers) is used, no comutagenesis is seen. This suggests that arsenite can inhibit excision repair in *E. coli*. Studies on other metal compounds are being planned.

## Mismatch Repair

Mismatch repair is a form of excision repair which operates preferentially on the newly synthesized daughter strand of DNA to remove replication errors. It differs from the previously described proofreading, which corrects replication errors only at the 3' terminus. Mismatch repair can be assayed in *E. coli* by the ability to convert  $\lambda$  heteroduplex DNA to the homoduplex (42). Genetic evidence suggests that the parental and daughter strand DNA can be distinguished by the presence of methyl groups on parental DNA and their absence in the newly synthesized daughter strands (43).

In mammalian cells, newly synthesized DNA is also undermethylated (44). Since mammalian cells have been shown to convert SV<sub>40</sub> heteroduplex DNA (45), mutation suppression by a methylation-instructed mismatch repair system is likely to exist in eukaryotic cells.

Agents which inhibit mismatch repair might act as mutagens by preventing correction of spontaneous replication errors, or as comutagens by preventing correction of mismatches due to DNA which has been altered by a mutagen. It would be of interest to determine the effects of metals on this system, perhaps by assaying the conversion of heteroduplex DNA. The enzymology of mismatch repair is not well understood (45). If a mammalian nuclease which specifically recognizes mismatches in DNA were identified, the effects of metals on this enzyme might be of interest.

## Other Pathways of Damage Correction

Recently, two alternative modes by which damaged DNA can be repaired have come to light. Neither of these modes involve cleavage of the phosphodiester bond and the subsequent repair replication characteristic of excision repair. Both involve only correction of the damaged base, and no studies on the effects of metals on these systems have been carried out.

When DNA is damaged by carcinogenic alkylating agents, a number of alkylation products are formed. One which is now thought to be of critical importance in carcinogenesis is O<sup>6</sup>-alkylguanine. In animal tissues, there is evidence that removal of O<sup>6</sup>-methylguanine from DNA can occur (46). This activity may be induced by prolonged exposure to alkylating agents. If there are enzymes which can remove a methyl group from O<sup>6</sup>-methylguanine, it is possible that other enzymes might exist which could remove other types of damage directly, without requiring the excision repair pathways.

A second recent finding involves an enzyme (sometimes called "insertase") which is able to insert a purine into apurinic sites in DNA (47). Apurinic sites can arise spontaneously, can be generated by chemical action, or can result from the action of an *N*-glycosylase. Base excision repair may be avoided by the reinsertion of a base at the AP site. The purine which is inserted also appears to be the correct one. However, the authors speculate that direct purine insertion might be more error-prone than an excision repair pathway (47). So far, there have been no reports of a pyrimidine insertase.

## Why Carcinogenic Metals Are Not Mutagenic in Microbial Systems

Unlike organic carcinogens, carcinogenic metals cannot be predicted with high accuracy in bacterial mutagenesis tests. Of the metals suspected or known to be carcinogenic, only chromate has given consistently positive results. Even in this case, chromate is a very weak mutagen which can best be detected in a fluctuation test rather than in standard agar plate assays (48). Reasons for the failure of bacterial mutagenesis tests to detect the mutagenicity of carcinogenic metals may be due to technical problems, such as precipitation of the metal in the medium commonly used. There are a number of other possibilities concerning the mutagenicity of carcinogenic metal compounds.

(1) As pointed out by Rosenkranz et al. (17), strongly bacteriocidal agents can obscure mutagenicity if the results are expressed as mutants/plate, without taking into account the survival level.

(2) Bacteria and mammalian cells may differ in their mutagenic response to metals. So far, few metal compounds have been tested in mammalian systems for mutagenicity, a subject which should have high priority.

(3) Carcinogenic metals may be comutagens rather than mutagens. Comutagenesis might occur by inhibition of (error-free) DNA repair pathways or by the formation of additional lesions on DNA by the combined action of metal plus mutagen.

(4) Bacterial strains in current use may be genetically incapable of giving a positive mutagenic response to metals. If, as suggested by Sirover and Loeb (16), carcinogenic metals cause infidelity in DNA synthesis, bacteria might be able to correct the errors by mismatch repair. Strains of bacteria lacking mismatch repair might be more suitable for studies on metal mutagenesis.

(5) Finally, it is altogether possible that there is no correlation between carcinogenicity and mutagenicity (or comutagenicity) of metals.

## REFERENCES

1. Miller, J., and Miller, E. Ultimate chemical carcinogens as reactive mutagenic electrophiles. In: *Origins of Human Cancer*, H. Hiatt, J. Watson, and J. Winston, Eds. Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1977, pp. 603-627.
2. Prakash, L., and Strauss, B. Repair of alkylation damage: Stability of methyl groups in *Bacillus subtilis* treated with methyl methane sulfonate. *J. Bacteriol.* 102: 760 (1970).
3. Setlow, R. Repair deficient human disorders and cancer. *Nature* 271: 713 (1978).
4. Vainio, H., and Sorsa, M. Chromosome aberrations and their relevance to metal carcinogenesis. *Environ. Health Perspect.* 40: 173 (1981).
5. Flessel, C. P. Metals as mutagenic initiators of cancer. In: *Trace Metals in Health and Disease*, N. Kharasch, Ed., Raven Press, New York, 1977, pp. 109-122.
6. Demeric, M., and Hanson, J. Mutagenic action of manganous chloride. *Cold Spring Harbor Symp. Qual. Biol.* 16: 215 (1951).
7. Nishioka, H. Mutagenic activities of metal compounds in bacteria. *Mutat. Res.* 31: 185 (1975).
8. Rossman, T. G., Stone, D., Molina, M., and Troll, W. Absence of arsenite mutagenicity in *E. coli* and Chinese hamster cells. *Environ. Mut.* 2: 371 (1980).
9. McCann, J., Choi, E., Yamasaki, E., and Ames, B. E. Detection of carcinogens as mutagens in the Salmonella-microsome test: Assay of 300 chemicals. *Proc. Natl. Acad. Sci. (U.S.)* 72:5135 (1975).
10. Workshop Report. *In vitro* models and methods for bioassay and studies of cellular mechanisms. *Environ. Health Perspect.* 40: 35 (1981).
11. Drake, J. W., and Baltz, R. H. The biochemistry of mutagenesis. *Ann. Rev. Biochem.* 45: 11 (1976).
12. Sinr, B., Fraenkel-Conrat, H., and Kusmierek, J. T. Preparation and template activities of polynucleotides containing O<sup>2</sup>- and O<sup>4</sup>-alkyluridine. *Proc. Natl. Acad. Sci. (U.S.)* 75: 1722 (1978).
13. Mukai, F., Hawryluk, I., and Shapiro, R. The mutagenic specificity of sodium bisulfite. *Biochem. Biophys. Res. Commun.* 39: 983 (1970).
14. Sundaralingam, V. S. M. The crystal structure of metal complexes of nucleic acids and their constituents. *Crit. Rev. Biochem.* 6: 245 (1979).
15. Eichhorn, G. L. *Inorganic Biochemistry*, Vol. 2. Elsevier, New York, 1973, pp. 1191-1240.
16. Sirover, M. A. and Loeb, L. A. Infidelity of DNA synthesis *in vitro*: screening for potential metal mutagens or carcinogens. *Science* 194: 1434 (1976).
17. Rosenkranz, H., Sutter, B., and Speck, W. T. Mutagenicity and DNA-modifying activity: a comparison of two microbial assays. *Mutat. Res.* 41: 61 (1976).
18. Kada, T., Sadate, Y., and Tutikawa, K. In vitro and host-mediated "rec-assay" procedures for screening chemical mutagens, and ploxine, a mutagenic red dye detected. *Mutat. Res.* 16: 165 (1972).
19. Painter, R. B. Rapid test to detect agents that damage human DNA. *Nature* 265: 650 (1977).
20. Luke, M. Z., Hamilton, L., and Hollaches, T. C. Beryllium-induced misincorporation by a DNA polymerase: a possible factor in beryllium toxicity. *Biochem. Biophys. Res. Commun.* 62: 497 (1975).
21. Byrnes, J. J., Downy, K. M., Que, B. G., Lee, Y. W., Black, V. L., and So, A. G. Selective inhibition of the 3' to 5' exonuclease activity associated with DNA polymerases: a mechanism of mutagenesis. *Biochemistry* 16: 3740 (1977).
22. Sirover, M. A., Dube, D. K., and Loeb, L. A. On the fidelity

- of DNA synthesis VIII. Metal activation of *E. coli* DNA polymerase I. *J. Biol. Chem.* 254: 107 (1979).
23. Boiteux, S., Villani, G., Spadari, S., Zambrano, F., and Radman, M. Making and correcting errors in DNA synthesis: In vitro studies of mutagenesis. In: DNA repair mechanisms. P. C. Hanawalt, E. C. Friedberg, and C. F. Fox, Eds. Academic Press, New York, 1978, pp. 73-84.
  24. Witkin, E. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. *Bacteriol. Rev.* 40: 869 (1976).
  25. Vallani, G., Boiteux, S., and Radman, M. Mechanism of ultraviolet-induced mutagenesis: Extent and fidelity of *in vitro* DNA synthesis of irradiated templates. *Proc. Natl. Acad. Sci. (U.S.)* 75: 3037 (1978).
  26. Rossman, T., Meyn, M. S. and Troll, W. Effects of sodium arsenite on the survival of UV-irradiated *Escherichia coli*: inhibition of a recA-dependent function. *Mutat. Res.* 30: 157 (1975).
  27. Rossman, T., Meyn, M. S., and Troll, W. Effects of arsenite on DNA repair in *Escherichia coli*. *Environ. Health Perspect.* 19: 229 (1977).
  28. Löfroth, G., and Ames, B. N. Mutagenicity of inorganic compounds in *Salmonella typhimurium*: arsenic, chromium and selenium (abstr.). *Mutat. Res.* 53: 65 (1978).
  29. Venitt, S., and Levy, L. Mutagenicity of chromates in bacteria and its relevance to chromate carcinogenesis. *Nature* 250: 493 (1974).
  30. Bockstahler, L. E. and Lytle, C. D. Radiation enhanced reactivation of nuclear replicating mammalian viruses. *Photochem. Photobiol.* 25: 477 (1977).
  31. Sarasin, A. R., and Hanawalt, P. C. Carcinogens enhance survival of UV-irradiated simian virus 40 in treated monkey kidney cells: Induction of a recovery pathway? *Proc. Natl. Acad. Sci. (U.S.)* 75: 346 (1978).
  32. Das Gupta, U. B., and Summers, W. C. Ultraviolet reactivation of herpes simplex virus is mutagenic and inducible in mammalian cells. *Proc. Natl. Acad. Sci. (U.S.)* 75: 2378 (1978).
  33. Cleaver, J. E. DNA repair and its coupling to DNA replication in eukaryotic cells. *Biochim. Biophys. Acta* 516: 489 (1978).
  34. Cleaver, J. E. Absence of interaction between x-rays and UV light in inducing ouabain- and thioguanine-resistant mutants in Chinese hamster cells. *Mutat. Res.* 52: 247 (1978).
  35. Stone-Wolff, D. S., and Rossman, T. G. Effects of inhibitors of de novo protein synthesis on UV-mutagenesis in Chinese hamster cells. Evidence against mutagenesis via inducible error-prone DNA repair. *Mutat. Res.*, in press.
  36. Kimball, R. F. The relation of repair phenomena to mutation induction in bacteria. *Mutat. Res.* 55: 85 (1978).
  37. Hanawalt, P. C., Friedberg, E. C. and Fox, C. F., Eds. *DNA Repair Mechanisms*, Academic Press, New York, 1978.
  38. Lehmann, A. R., and Bridges, B. A. DNA repair. *Essays Biochem.* 17: 71 (1977).
  39. Hewitt, R. R., and Meyn, R. E. Applicability of bacterial models of DNA repair and recovery to UV-irradiated mammalian cells. *Adv. Radiat. Biol.* 7: 153 (1978).
  40. McCormick, J. J., and Maher, V. M. Mammalian cell mutagenesis as a biological consequence of DNA damage. In: *DNA Repair Mechanisms*, P. C. Hanawalt, E. C. Friedberg, and C. F. Fox, Eds., Academic Press, New York, 1978.
  41. Rossman, T. G. Enhancement of UV-mutagenesis by low concentrations of arsenite in *E. coli*. *Mutat. Res.*, in press.
  42. Nevers, P., and Spatz, H. *Escherichia coli* mutants uvr D and uvr E deficient in gene conversion of  $\lambda$  heteroduplexes. *Mol. Gen. Genet.* 139: 233 (1975).
  43. Glickman, B., van den Elsen, P., and Radman, M. Induced mutagenesis in *dam*<sup>-</sup> mutants of *Escherichia coli*: a role for O<sup>6</sup>-methyladenine in mutation avoidance. *Mol. Gen. Genet.* 163: 307 (1978).
  44. Adams, R. Newly synthesized DNA is not methylated. *Biochem. Biophys. Acta* 335: 365 (1974).
  45. Radding, C. M. Genetic recombination: strand transfer and mismatch repair. *Ann. Rev. Biochem.* 47: 847 (1978).
  46. Pegg, A. E. Enzymatic removal of O<sup>6</sup>-methylguanine from DNA by mammalian cell extracts. *Biochem. Biophys. Res. Commun.* 84: 166 (1978).
  47. Deutsch, W. A., and Linn, S. DNA binding activity from cultured human fibroblasts that is specific for partially depurinated DNA and that inserts purines into apurinic sites. *Proc. Natl. Acad. Sci. (U.S.)* 76: 141 (1979).
  48. Nestmann, E. F., Matula, T. I., Douglas, G. R., Bora, K. C., and Kowbel, D. J. Detection of the mutagenic activity of lead chromate using a battery of microbial tests. *Mutat. Res.* 66: 357 (1979).